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NEWS	5	NOV 30	PHAR reloaded with additional data
NEWS	6	DEC 01	LISA now available on STN
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NEWS	8	DEC 15	MEDLINE update schedule for December 2004
NEWS	9	DEC 17	ELCOM reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	10	DEC 17	COMPUAB reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	11	DEC 17	SOLIDSTATE reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	12	DEC 17	CERAB reloaded; updating to resume; current-awareness alerts (SDIs) affected
NEWS	13	DEC 17	THREE NEW FIELDS ADDED TO IFIPAT/IFIUDB/IFICDB
NEWS	14	DEC 30	EPFULL: New patent full text database to be available on STN
NEWS	15	DEC 30	CAPLUS - PATENT COVERAGE EXPANDED
NEWS	16	JAN 03	No connect-hour charges in EPFULL during January and February 2005
NEWS	17	FEB 25	CA/CAPLUS - Russian Agency for Patents and Trademarks (ROSPATENT) added to list of core patent offices covered
NEWS	18	FEB 10	STN Patent Forums to be held in March 2005
NEWS	19	FEB 16	STN User Update to be held in conjunction with the 229th ACS National Meeting on March 13, 2005
NEWS	20	FEB 28	PATDPAFULL - New display fields provide for legal status data from INPADOC
NEWS	21	FEB 28	BABS - Current-awareness alerts (SDIs) available
NEWS	22	FEB 28	MEDLINE/LMEDLINE reloaded
NEWS	23	MAR 02	GBFULL: New full-text patent database on STN
NEWS	24	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	25	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	26	MAR 22	KOREAPAT now updated monthly; patent information enhanced
NEWS	27	MAR 22	Original IDE display format returns to REGISTRY/ZREGISTRY
NEWS	28	MAR 22	PATDPASPC - New patent database available
NEWS	29	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags

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NEWS HOURS	STN Operating Hours Plus Help Desk Availability
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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 13:51:31 ON 22 MAR 2005
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7 FILES IN THE FILE LIST

=> s oligo-dt and VN###
L1 13 OLIGO-DT AND VN###

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)

=> d ibib abs l2 1-7

L2 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23088 BIOTECHDS
TITLE: Comparing gene expression profiles of two or more samples, by using sample-specific primers for cDNA synthesis and amplification of the synthesized cDNAs, and comparing levels of abundance of genes between samples;
expression profiling and DNA primer useful for drug screening and diagnosis
AUTHOR: SLEPNEV V I
PATENT ASSIGNEE: SENTION
PATENT INFO: US 2003077611 24 Apr 2003
APPLICATION INFO: US 2002-113034 1 Apr 2002
PRIORITY INFO: US 2002-113034 1 Apr 2002; US 2001-346140 24 Oct 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-625538 [59]
AN 2003-23088 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Comparing gene expression profiles of two or more samples, comprising synthesizing several first strand cDNAs from first sample (S1) using oligonucleotide primer having sample-specific sequence tag, selectively amplifying subset of cDNA, detecting abundance of amplified products, where abundance determines expression profile (EP) of genes in S1, and comparing EP of genes in S1 with EP of genes in second sample, is new.

DETAILED DESCRIPTION - Comparing (M1) gene expression profiles of two or more samples, comprises: (a) synthesizing a number of first strand cDNAs from a first sample using a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal; (b)

selectively amplifying at least a subset of the cDNA so as to generate one or more sample specific amplified products; (c) detecting the abundance of one or more sample specific amplified products, where the abundance determines an expression profile of one or more genes in the first sample; and (d) comparing the expression profile of the genes in the first sample with an expression profile of genes in a second sample, where a difference in the expression profile indicates differential expression of the genes in the two samples. INDEPENDENT CLAIMS are also included for: (1) identifying (M2) a modulator which regulates one or more gene expression in a sample, by performing M1, where the expression profile of one or more genes in S1 is compared before contacting with the modulator, with an expression profile of one or more genes in S1 after contacting the modulator, where a difference in the expression profile indicates that the modulator regulates gene expression in the sample;(2) a composition (I) for detecting the level of gene expression, comprises a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal; and (3) a kit (II) for detecting the level of gene expression, comprises a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal, and packaging material.

WIDER DISCLOSURE - Disclosed is a business method to conduct a pharmaceutical business.

BIOTECHNOLOGY - Preferred Method: In M1, the first oligonucleotide primer comprises at least one degenerate nucleotide. The sample-specific sequence tag comprises at least one artificial nucleotide. Synthesizing the first strand cDNAs comprises reverse transcribing RNA from two or more sample sources into first strand cDNA, where the cDNA is differentially tagged according to the sources. The first strand cDNAs are synthesized by reverse transcription using total RNAs or mRNAs derived from the first sample. A third oligonucleotide primer comprising the sequence-specific sequence tag of the first oligonucleotide primer is used for the amplifying so as to generate one or more sample-specific amplified products. The sample-specific sequence in the first oligonucleotide primer is 15-30 nucleotides, preferably 20-24 nucleotides in length. The first oligonucleotide primer further comprises a sequence of 5' **oligo(dT)n VN** 3', where n is at least 5, V is dATP, dGTP or dCTP, and N is dTTP (or dUTP), dATP, dGTP or dCTP. The first oligonucleotide primer is provided as a mixture of primers comprising (5'-(specific sequence tag)20-24T12-16AN-3', 5'-(specific sequence tag)20-24T12-16CN-3', and 5'-(specific sequence tag)20-24T12-16GN-3'), where the specific sequence tags are identical or different for each primer in the mixture. The first oligonucleotide primer and the sample-specific sequence tag is located at the 5' of **oligo(dT)**nVN. M1 further comprising synthesizing one or more second strand cDNAs complementary to the first strand cDNAs using a second oligonucleotide primer comprising a first arbitrary sequence tag, and amplifying at least a subset of the second strand cDNAs so as to generate one or more sample specific amplified products. The second oligonucleotide primer further comprises a second sequence which is complementary to a subset of the first strand cDNAs so as to permit the synthesis of one or more second strand cDNAs. The second oligonucleotide primer is located 3' of the first arbitrary sequence. The second oligonucleotide further comprises a sequence of (Z)m between the first and second sequences, where Z is a nucleotide which can form base pair with any of A, T, G or C, and m is at least 2, preferably m is 4. The second sequence is 5-10, preferably 6-7 nucleotides in length. The arbitrary sequence within the second oligonucleotide primer comprises a A-T rich region and a G-C rich region which is located at 5' of the A-T rich region. The second oligonucleotide primer used is the same for the two or more samples to be compared. The amplification is performed by PCR. The amplifying further comprises using a fourth oligonucleotide primer which comprises the first arbitrary sequence tag of the second

oligonucleotide primer. The fourth oligonucleotide primer used is the same for the two or more samples to be compared. The second sequence within the second oligonucleotide prime is gene-family-specific, a peptide specific for a protein family, or a sequence encoding a signature sequence motif for a specific protein family. The protein family is selected from receptor tyrosine kinases, G protein coupled receptors, seven transmembrane receptors, ion channels, cytokine receptors, tumor markers, mitogen associated protein kinase (MAPK) cascade kinases, transcriptional factors, GTPases, ATPases, and development protein markers. The first strand cDNA is synthesized in a solution without attaching to a solid support, or by attaching to a solid support. The solid support is a microparticle or an inner wall of a reaction tube. M1 further comprises separating the second strand cDNA from the first strand cDNA before amplifying the second strand cDNAs. The third oligonucleotide primer is linked to a detectable label (selected from fluorescent labels, radioactive labels, colorimetrical labels, magnetic labels, and enzymatic labels) or is labeled with a sample-specific label. The amplified products are sampled at a predetermined time or cycle interval during the amplification. The abundance is detected for each sampled amplified product. M1 further comprises separating the amplified products before detecting the abundance of the amplified products. The amplified products are separated and their abundance is detected by chromatography, mass spectrometry, measurement of fluorescence, or measurement of optical density. The amplified products are separated by electrophoresis, preferably capillary electrophoresis. The difference in the expression profile of the genes is measured by a ratio of sample-specific detectable labels on amplified products from the genes between two or more samples. M1 further comprises generating an amplification plot, calculating a Ct of amplification for each of the genes, and measuring the difference in the expression profile by a ratio of the Cts. M1 further comprises collecting one or more genes which are differentially expressed and identifying the sequence identities of the genes by DNA sequencing.

USE - M1 is useful for comparing gene expression profiles of two or more samples chosen from normal sample, disease sample, sample at a given development stage or condition, sample prior to a given treatment stage or condition, and a sample at a given culturing stage or condition. The samples are derived from an animal, an organ, tissue type and a cell type. M2 is useful for identifying a modulator which regulates one or more gene expressions in a sample. (I) or (II) is useful for detecting the level of gene expressions (all claimed). M1 is useful for studying normal developmental processes, for identifying potential drug targets for further research and development and useful diagnosis marker for certain pathological conditions, to study the effects of certain treatments on cells, tissues or individual, or for characterization of cells or organisms that underwent genetic modification.

EXAMPLE - RNA was prepared. Tissues were homogenized in a homogenizer at 1 ml of TRIZOL reagent per 50-100 mg of tissue of 30 seconds, followed by a final homogenization of 1 minute. The homogenized tissues were left for at least 15 minutes up to an hour, at room temperature, or they were stored in -70degreesC until needed. 0.2 ml of chloroform per ml of Trizol was added and mixed by shaking. The mixture was incubated at room temperature for 5 minutes, then centrifuged for 5 minutes. The upper phase was collected into a separate tube. 0.5 ml of isopropyl alcohol per ml of Trizol was added to precipitate RNA. The mixture was put on ice for 5 minutes and was centrifuged for 10 minutes. The supernatant was removed and the pellet was washed with 1 ml 75% EtOH per ml of Trizol, mixed, and centrifuged for 5 minutes. The supernatant was moved and the pellet was air dried for 30 minutes to 1 hour. After pellet was air dried, the pellet was resuspended in RNAse free water to a desired concentration. The RNA extracted was cleaned up by adding the appropriate volume of buffer RLT, and mixed thoroughly. An appropriate volume of ethanol (96-100%) was added to the diluted RNA and mixed thoroughly by shaking vigorously. The sample was applied to an RNeasy midi spin column or RNeasy maxi spin column and was placed in a 15 ml or

50 ml centrifuge tube and centrifuged. The flow-through was discarded. Reverse transcription in solution was performed. The RNA samples (1-5 mul) were mixed with 1 mul of dNTPs solution (10 mM) and 0.0005-0.5 muM (final concentration in 20 mul mixture) of first oligonucleotide, heated for 7 minutes at 70degreesC, and cooled for 2 minutes at 4degreesC. The above mixture was then mixed with the reaction mixture (4 mul reverse transcriptase (RT) buffer (250 mM Tris-HCl) (pH 8.3 at 25degreesC), 375 mM KCl, 15 mM MgCl2, 2 mul 0.1 M dithiothreitol (DTT), 1 mul RNAse inhibition and 1 mul of SuperScriptII reverse transcriptase and 5-10 mul of water) in a total volume of 20 mul. The reverse transcription reaction was incubated for 1-2 hours at 45degreesC and was terminated by heating at 65degreesC for 10 minutes. An aliquot of sample (5-20 mul) was directly analyzed by PCR. Optionally, the RNA templates were degraded by incubation with RNAse H enzyme prior to PCR amplification. Second strand synthesis was performed. The synthesis of the second strand of bound DNA was performed with mixture of Taq polymerase (0.5-1.5 microns) and Pwo DNA polymerase in PCR thermocycler. The reaction mixture included 6-10 mul of cDNA on oligobeads from RT reaction, 5 mul of 10xPCR buffer or 10xRT-PCR buffer (500 mM Tris, 200 mM KCl, 100 mM (NH4)2SO4, 2.5 mM Mg2Cl, pH 8.5), 0.1 mM dNTPs, 0.5-1 mul of second primer (100 muM) in a total volume of 50 mul. The synthesized second DNA strand was removed from the beads at 96degreesC and used for further amplification. PCR amplification of synthesized cDNA (5-20 mul) was performed in the presence of 10 mul of 10xPCR buffer of 10xRT-PCR buffer, 2-3 mM MgCl2, 0.05-0.2 mM dNTPs, 0.1-1 muM of third primers labeled with either FAM, Rox, or Hex, 0-1 muM of unlabeled third primers, 1-2 mul of fourth primer, 0-5% dimethylsulfoxide (DMSO), 0.5-2 microns of proofreading DNA polymerase (Pwo or Tgo) and 1.5-3 microns of hot-start DNA polymerase. The amplification was conducted using I-cycler or PCR Express. Aliquots of samples (typically 25 mul) were withdrawn after each cycle at the end of extension step (72degreesC) starting with 10-15th cycle. Equal volume of PCR mix containing primers, polymerase and dNTPs was placed into reaction mix after each sample removal. The collected samples were analyzed. Capillary electrophoresis was performed on SCE 9610 fully automated 96-capillary electrophoresis genetic analysis system. (40 pages)

L2 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17928 BIOTECHDS
TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a **oligo-dT** primer of a specific sequence and a detectable marker at its 5' end;
gene expression identification an characterization, database and computer bioinformatic software
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC
PATENT INFO: WO 2002036828 10 May 2002
APPLICATION INFO: WO 2000-US45401 1 Nov 2000
PRIORITY INFO: US 2000-244933 1 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-508123 [54]
AN 2002-17928 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific **oligo-dT** primer sequence, where the identimer comprises a detectable marker at its 5' end.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an

identimer comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the **VNx** portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-**VNx** (I) Tn-**VNNN** (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource

efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L2 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002389129 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12137780
TITLE: Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions.
AUTHOR: Lekanne Deprez Ronald H; Fijnvandraat Arnoud C; Ruijter Jan M; Moorman Antoon F M
CORPORATE SOURCE: Experimental and Molecular Cardiology Group, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.. r.h.lekanne@amc.uva.nl
SOURCE: Analytical biochemistry, (2002 Aug 1) 307 (1) 63-9. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20020725
Last Updated on STN: 20030211
Entered Medline: 20030210

AB The recent development of real-time PCR has offered the opportunity of sensitive and accurate quantification of mRNA levels that is crucial in biomedical research. Although reverse transcription (RT)-PCR is at present the most sensitive method available, many low abundant mRNAs are, although detectable, often not quantifiable. Here we report an improved two-step real-time RT-PCR procedure using SYBR green I and the LightCycler that better permits accurate quantification of mRNAs. Omission of dithiothreitol from the cDNA synthesis reaction was found to be crucial. This resulted in a lower cycle number at which the cDNA level is determined (C(T) value), steeper amplification curves, and removal of background fluorescence in the subsequent PCR. In addition, the choice of the cDNA priming oligo can improve detection sensitivity even further. In contrast to hexamer primer usage, both gene-specific and **oligo-dT(VN)** priming were very efficient and accurate, with gene-specific priming being the most sensitive. Finally, accurate quantification of mRNAs by real-time PCR using SYBR green I requires verification of the specificity of PCR by both melting curve and gel analysis.

L2 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:519205 BIOSIS
DOCUMENT NUMBER: PREV200100519205
TITLE: Comparison of probe labeling methods for cDNA microarray studies.
AUTHOR(S): Modrusan, Zora [Reprint author]; Young, Laura S. [Reprint author]; Cisneros, Yvette G. [Reprint author]; Reynolds, Mark A. [Reprint author]
CORPORATE SOURCE: Microarray Systems, Incyte Genomics, Fremont, CA, USA
SOURCE: International Genome Sequencing and Analysis Conference,

(2000) Vol. 12, pp. 80. print.
Meeting Info.: 12th International Genome Sequencing and Analysis Conference. Miami Beach, Florida, USA. September 12-15, 2000.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Nov 2001
Last Updated on STN: 23 Feb 2002

AB DNA microarrays have been a vital tool in populating gene expression databases. Competitive hybridization between two differentially labeled probe populations was used to obtain such information on Incyte's Gene Expression Microarrays (GEMs). cDNA probes were generated from 200 ng mRNA using fluorescently labeled random nonamers (GEMBrightTM). Probe profiling on PAGE and fluorescence quantitation was used to determine the efficiencies of Cy3 and Cy5 dye incorporation. These methods were developed, in part, to prevent the use of failed probe reactions. Performance of GEMBrightTM probes was compared to a commonly used DNA microarray probe labeling method, **oligo dT** priming. Probes generated with dT17 and dT17 (**VN**) primers were characterized similar to GEMBrightTM probes. Following GEM hybridizations, a non-specific signal mediated by yeast control fragments was observed in the presence of standard blocking reagents. Similar results were also observed with another **oligo dT** priming method, the 3DNATM Expression Array Detection Kit from Genisphere. This study shows performance characteristics of the GEMBrightTM labeling system and demonstrates that a careful consideration of probe labeling methods is essential for populating gene expression databases.

L2 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1999141232 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9973624
TITLE: Increased specificity of reverse transcription priming by trehalose and oligo-blockers allows high-efficiency window separation of mRNA display.
AUTHOR: Mizuno Y; Carninci P; Okazaki Y; Tateno M; Kawai J; Amanuma H; Muramatsu M; Hayashizaki Y
CORPORATE SOURCE: Laboratory for Genome Exploration Research Project, Genomic Sciences Center (GSC), Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074, Japan.
SOURCE: Nucleic acids research, (1999 Mar 1) 27 (5) 1345-9.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 19990504
Entered Medline: 19990422

AB We have developed a method for high-efficiency window separation of cDNA display by increasing the specificity of priming in reverse transcription. In the conventional method, two-base anchored **oligo(dT**) primers (5'dT16VN3', where N is any base and V is G, A or C) are used to make windows for the display of transcripts. However, reverse transcriptase often extends misprimed oligonucleotides. To avoid mispriming from dT16VN primers, we have developed two new technologies. One is higher temperature priming with reverse transcriptase thermoactivated by the disaccharide trehalose. The other is the use of competitive oligonucleotide blockers that hybridize to the non-selectively primed mRNAs, preventing the mispriming from the **VN** site. These methods were combined to improve restriction landmark cDNA scanning

(RLCS), resulting in the elimination of the redundant signals that appear in different windows. This was achieved by the increased specificity of initiation of reverse trans-cription from the beginning of poly(A) sites. This method paves the way for the precise visualization of transcripts to allow expression profiles in individual tissues and at each developmental stage to be understood.

L2 ANSWER 6 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 97:113853 SCISEARCH
THE GENUINE ARTICLE: WE924
TITLE: DD/AP-PCR: Combination of differential display and arbitrarily primed PCR of **oligo(dT)** cDNA
AUTHOR: Rothschild C B (Reprint); Brewer C S; Bowden D W
CORPORATE SOURCE: WAKE FOREST UNIV, BOWMAN GRAY SCH MED, DEPT BIOCHEM, WINSTON SALEM, NC 27157 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: ANALYTICAL BIOCHEMISTRY, (1 FEB 1997) Vol. 245, No. 1, pp. 48-54.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0003-2697.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 11

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this report we describe the first direct comparison of differential display (DD) and arbitrarily primed PCR (AP-PGR) amplification of **oligo(dT)**-primed cDNA. Our results indicate that both of these widely used RNA fingerprinting techniques have their respective advantages and limitations. DD produces profiles specific to the anchored **oligo(dT)** primer used for cDNA synthesis. AP-PCR displays significant redundancy of profiles generated from different **oligo(dT)** cDNA pools, but is not as biased to the isolation of AT-rich or 3' sequences. It was found that both techniques can utilize cDNA synthesized using a generic anchored **oligo(dT)** primer (dT(12)**VN**; equimolar amounts of dT(12)VA, dT(12)VC, dT(12)VG, and dT(12)VT, where V is dA, dC, or dG); this efficiently selects for poly(A)(+) sequences from total RNA, and significantly reduces the number of cDNA preparations required per experiment. Using dT(12)**VN** cDNA pools generated from rat liver, spleen, and brain, the two approaches (AP-PCR and DD) were used in combination. Several known mRNAs were identified; some were unique to either technique and some were common to both. Since it is the RNA which is usually the limiting resource, maximum utilization may be achieved by generating a single pool of dT(12)**VN**-primed cDNA and performing both AP-PCR and DD (DD/AP-PCR). (C) 1997 Academic Press.

L2 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1995:52095 CAPLUS
DOCUMENT NUMBER: 122:232159
TITLE: Isolation and nucleotide sequence analysis of a partial cDNA clone for potato virus Y-**VN** (Korean isolate) genome
AUTHOR(S): Cho, Hye Sun; Jun, Se Il; Kwon, Seok Yoon; Park, Eun Kyung; Paek, Kee Yoeup; Paek, Kyung-Hee
CORPORATE SOURCE: Genetic Eng. Res. Inst., KIST, Taejon, 305-600, S. Korea
SOURCE: Molecules and Cells (1994), 4(2), 143-8
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Potato virus Y-**VN** (PVY-**VN**) isolated from Nicotiana tabacum var. "Burley 21" in Korea was verified by electron microscopy observations of purified virions and SDS-PAGE anal. of viral proteins. RNA was isolated from the purified PVY particles and about 10 μg of RNA was obtained from approx. 500 μg of particles. Double strand cDNA was synthesized by using **oligo(dT)** primer and cloned in a SmaI site of pUC19. The size of cDNA inserts was estimated by restriction enzyme digestion analyses. The identification of coat protein gene of PVY-**VN** was done by Southern blot anal. using a radiolabeled 900 bp fragment of PVY (isolate Amigo) as a probe. The insert of the longest cDNA (clone 18) was subcloned into pUC19 and subjected to sequencing anal. The nucleotide sequences of clone 18 reported here comprise the entire viral coat protein gene and almost the full length of the nuclear inclusion protein b (NIb) gene region, totalling 2,541 nucleotides in length. The PVY-**VN** coat protein (CP) starts at G1,408 and codes for 267 amino acids with Mr = 30,062. At the amino acid level the CP sequence of PVY-**VN** strain is 93 to 96% homologous with those PVY strains whose CP sequences are known.

=> differential display and VN###
DIFFERENTIAL IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s differential display and VN###
L3 20 DIFFERENTIAL DISPLAY AND VN###

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)

=> d ibib abs l4 1-8

L4 ANSWER 1 OF 8 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2004124362 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15015574
TITLE: In-silico analysis of kallikrein gene expression in pancreatic and colon cancers.
AUTHOR: Yousef George M; Borgono Carla A; Popalis Cynthia; Yacoub George M; Polymeris Mary-Ellen; Soosaipillai Antoninus; Diamandis Eleftherios P
CORPORATE SOURCE: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada.
SOURCE: Anticancer research, (2004 Jan-Feb) 24 (1) 43-51. Journal code: 8102988. ISSN: 0250-7005.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 20040313
Last Updated on STN: 20040522
Entered Medline: 20040521

AB Human kallikreins are a cluster of 15 serine protease genes located in the chromosomal band 19q13.4, a non-randomly rearranged region in many solid tumors, including pancreatic cancer. We utilized the SAGE and EST databases of the Cancer Genome Anatomy Project to perform in-silico analysis of kallikrein gene expression in normal and cancerous pancreatic and colon tissues and cell lines using virtual Northern blotting (**VNB**), digital **differential display** (DDD) and X-profiler. At least two kallikreins, KLK6 and KLK10, are significantly up-regulated in pancreatic cancer. We probed 2 normal and 6 pancreatic

cancer SAGE libraries with gene-specific tags for each of these kallikreins. KLK6 was found to be expressed in 5/6 cancer libraries and showed the most marked (5-fold) increase in average expression levels in cancer vs. normal. These data were verified by screening the EST databases, where all mRNA clones isolated were from cancerous libraries, with no clones detected in normal pancreatic tissues or cell lines. X-profiler comparison of two pools of normal and cancerous pancreatic libraries further verified the significant increase of KLK6 expression levels in pancreatic cancer. DDD data showed a 13-fold increase in KLK10 expression in pancreatic cancer. Three kallikrein genes, KLK6, 8 and 10 are overexpressed in colon cancer compared to normal colon, while one kallikrein, KLK1, is down-regulated. While no expression of KLK6 was detected in normal colon, KLK6-specific tags were detectable in 2 cancer libraries. Similar results were obtained by EST screening; no KLK6 clones were detected in any of the 28 normal libraries examined, while 10 KLK6 EST clones were found in colon adenocarcinoma. KLK10 was not detectable in normal colon. Gene-specific tags were, however, detectable with high density in colon cancer and 7 EST clones were found to be expressed in colon Adenocarcinoma.

L4 ANSWER 2 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-01800 BIOTECHDS
TITLE: Identifying target epitope or antigen comprises measuring cross reactivity of cytotoxic T cells specific for gene product differentially expressed by a cell which induces cytotoxic T cells;
disease specific target epitope identification and virus vector for use in disease therapy and gene therapy
AUTHOR: ZAUDERER M
PATENT ASSIGNEE: ZAUDERER M
PATENT INFO: US 2003133917 17 Jul 2003
APPLICATION INFO: US 1997-935377 22 Sep 1997
PRIORITY INFO: US 1997-935377 22 Sep 1997; US 1997-935377 22 Sep 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-829629 [77]
AN 2004-01800 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Identifying (M1) a target epitope or antigen, comprising providing cytotoxic T cells specific for gene product differentially expressed by cell which express the target epitope, and measuring cross reactivity of the cytotoxic T cells for the cell in which target epitopes are identified as gene product which induces cytotoxic T cells, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a viral vector (I) containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element; and (2) a vaccinia viral vector (II) containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element where the DNA insert encodes a target epitope identified by (M1) or a tumor specific target epitope.

BIOTECHNOLOGY - Preferred Method: In (M1), a modified **differential display** method is employed that increases resolution of DNA fragments and reduces the frequency of false positives. (M1) further involves the use of DNA fragments to isolate longer gene products following solution hybridization to single strand circles rescued from a phagemid DNA library. In (M1), the target epitope is specific to a cell infected with a virus, fungus, Mycobacteria or an autoimmune disease. Preferred Viral Vector: (I) is constructed by trimolecular recombination and is a vaccinia viral vector. (I) is derived by recombination with plasmid p7.5/tk or its derivatives or only

recombination with plasmid ptl/tk or its derivatives.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Vaccine. No biological data given.

USE - (M1) is useful for identifying target epitope or antigen, e.g., tumor specific target epitope or antigen which involves providing cytotoxic T cells specific for a gene product differentially expressed by a tumor cell which express the target epitope and measuring cross reactivity of the cytotoxic T cells for the tumor cell in which target epitopes are identified as the gene product which induces cytotoxic T cells. The tumor cell is derived from a single immortalized, non-tumorigenic cell line. The assay is performed on a panel of tumor cell lines each derived independently from a single non-tumorigenic cell. The generated cytotoxic T cells which react to tumor cells do not react to non-tumorigenic T cells (all claimed). A vaccinia viral vector (II) is useful as a vaccine for preventing or treating tumors in mammals, including humans. (M1) is also useful for identifying target antigens in other target cells against which it is desirable to induce cell-mediated immunity. (II) is also used for booster immunization subsequent to primary immunization.

ADMINISTRATION - (II) is administered orally, intradermally, intramuscularly, intraperitoneally, intravenously, sub-cutaneously, intranasally, transdermally or rectally. No dosage given.

ADVANTAGE - (M1) identifies potential antigens expressed not only by the pathogen but also by the host cell where gene expression is altered as a result of infection.

EXAMPLE - Vaccinia virus transfer plasmid pJ/K, a pUC13 derived plasmid with a vaccinia virus thymidine kinase gene containing an in-frame NotI site were modified to incorporate a strong vaccinia virus promoter followed by NotI and ApaI restriction sties. Two different vectors, p7.5/tk and pEL/tk, comprising either the 7.5K vaccinia virus promoter or a strong synthetic early/late (E/L) promoter were produced. The ApaI site was preceded by a strong translational initiation sequence including the ATG codon. This modification was introduced within the vaccinia virus thymidine kinase (tk) gene so that it was flanked by regulatory and coding sequences of the viral tk gene. The modifications with tk gene of these two new plasmid vectors were transferred by homologous recombination in the flanking tk sequences into the genome of the Vaccinia Virus WR strain derived **vNotI**-vector to generate new viral vectors v7.5/tk and vEL/tk. Two large viral DNA fragments were isolated each including a separate non-homologous segment of the vaccinia tk gene and together comprising all the genes required for assembly of infectious viral particles. Recombinant vaccinia virus were generated by homologous recombination. A further significant increase in the frequency of viral recombinants was obtained by transfection of Fowl Pox Virus (FPV) infected cells with a mixture of recombinant plasmids and the two large approximately 80 kilobases and 100 kilobases fragments of vaccinia virus v7.5/tk DNA produced by digestion with NotI and ApaI restriction endonucleases. Because the NotI and ApaI sites have been introduced into the tk gene, each of these large vaccinia DNA arms includes a fragments of the tk gene. Since there is no homology between the two tk gene fragments, the only way the two vaccinia arms can be linked was by bridging through the homologous tk sequences that flank the inserts in the recombinant transfer plasmid. The results showed that greater than 99 % of infectious vaccinia virus produced in triply transfected cells was recombinant for a DNA insert. The tri-molecular recombination strategy yielded close to 100 % viral recombinants. (47 pages)

L4 ANSWER 3 OF 8 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2001:873447 SCISEARCH

THE GENUINE ARTICLE: 485YP

TITLE: Detection of an mRNA polymorphism by **differential display**

AUTHOR: Liang S (Reprint); Rossby S P; Liang P; Shelton R C;

Manier D H; Chakrabarti A; Sulser F
CORPORATE SOURCE: Vanderbilt Univ, Med Ctr, Dept Cell Biol, Nashville, TN 37232 USA; Vanderbilt Univ, Med Ctr, Dept Psychiat, Nashville, TN 37232 USA; Vanderbilt Univ, Med Ctr, Dept Pharmacol, Nashville, TN 37232 USA
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR BIOTECHNOLOGY, (OCT 2001) Vol. 19, No. 2, pp. 121-124.
Publisher: HUMANA PRESS INC, 999 RIVERVIEW DRIVE SUITE 208, TOTOWA, NJ 07512 USA.
ISSN: 1073-6085.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 5
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Differential Display** (DD) technology was utilized to compare programs of gene expression in primary cultures of human skin fibroblasts from normal volunteers and patients diagnosed with melancholic depression. Polymorphic transcripts of a single gene differing by one tandem repeat sequence of four nucleotides (TGAT) in the 3' noncoding region were detected.

L4 ANSWER 4 OF 8 MEDLINE on STN
ACCESSION NUMBER: 2000218682 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10753647
TITLE: Metallothionein-null mice express altered genes during development.
AUTHOR: Kimura T; Oguro I; Kohroki J; Takehara M; Itoh N; Nakanishi T; Tanaka K
CORPORATE SOURCE: Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6, Yamada-oka, Suita, Osaka, 565-0871, Japan.
SOURCE: Biochemical and biophysical research communications, (2000 Apr 13) 270 (2) 458-61.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000508

AB Metallothionein (MT) can modulate transcriptional activity in vitro. We examined whether the absence of MT affects gene expression in vivo. We compared the hepatic RNA profiles of wild-type and MT-null neonatal mice using improved **differential display.** The hepatic MT level was maximal during neonatal development. We identified five cDNA fragments that were expressed in MT-null mice at different levels from those in wild-type mice. Two were fragments of MT-I and mutant MT-I cDNA. The sequences of the other cDNA fragments were identical to those of contrapsin, transketolase, and vanin-3. The latter two were up-regulated, whereas contrapsin was down-regulated in neonatal MT-null mice. These mRNA levels were remarkably different between the two strains of neonatal mice. Further characterization of the regulated mRNA identified here will determine whether or not they are primary or secondary effects of an MT deficiency.

L4 ANSWER 5 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
ACCESSION NUMBER: 1999:187773 BIOSIS
DOCUMENT NUMBER: PREV199900187773
TITLE: Increased specificity of reverse transcription priming by

trehalose and oligo-blockers allows high-efficiency window separation of mRNA display.

AUTHOR(S): Mizuno, Yosuke; Carninci, Piero; Okazaki, Yasushi; Tateno, Minako; Kawai, Jun; Amanuma, Hiroshi; Muramatsu, Masami; Hayashizaki, Yoshihide [Reprint author]

CORPORATE SOURCE: Laboratory for Genome Exploration Research Project, Genomic Sciences Center (GSC) and Genome Science Laboratory, Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba, Ibaraki, 305-0074, Japan

SOURCE: Nucleic Acids Research, (March 1, 1999) Vol. 27, No. 5, pp. 1345-1349. print.
CODEN: NARHAD. ISSN: 0305-1048.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 5 May 1999
Last Updated on STN: 5 May 1999

AB We have developed a method for high-efficiency window separation of cDNA display by increasing the specificity of priming in reverse transcription. In the conventional method, two-base anchored oligo(dT) primers (5'dT16VN3', where N is any base and V is G, A or C) are used to make windows for the display of transcripts. However, reverse transcriptase often extends misprimed oligonucleotides. To avoid mispriming from dT16VN primers, we have developed two new technologies. One is higher temperature priming with reverse transcriptase thermoactivated by the disaccharide trehalose. The other is the use of competitive oligonucletide blockers that hybridize to the non-selectively primed mRNAs, preventing the mispriming from the **VN** site. These methods were combined to improve restriction landmark cDNA scanning (RLCS), resulting in the elimination of the redundant signals that appear in different windows. This was achieved by the increased specificity of initiation of reverse trans-cription from the beginning of poly(A) sites. This method paves the way for the precise visualization of transcripts to allow expression profiles in individual tissues and at each developmental stage to be understood.

L4 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 97463936 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9322637

TITLE: Identification of gestationally regulated genes in rat myometrium by use of messenger ribonucleic acid **differential display.**

AUTHOR: Chien E K; Tokuyama Y; Rouard M; Phillippe M; Bell G I

CORPORATE SOURCE: Department of Obstetrics and Gynecology, University of Chicago, IL 60637, USA.

CONTRACT NUMBER: DK-20595 (NIDDK)
FD-R-001114 (FDA)
HD-22063 (NICHD)

SOURCE: American journal of obstetrics and gynecology, (1997 Sep) 177 (3) 645-52.
Journal code: 0370476. ISSN: 0002-9378.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 199710

ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971028

AB OBJECTIVE: We hypothesized that the proteins contributing to myometrial changes during gestation could be identified indirectly by analyzing the changing pattern of messenger ribonucleic acid expression in the myometrium during pregnancy. STUDY DESIGN: Ribonucleic acid was extracted from myometrium of timed pregnant Sprague-Dawley rats on days 12, 16, 20, 21, and 22 of pregnancy and on day 1 post partum. The technique of

messenger ribonucleic acid **differential display**, a simple and sensitive polymerase chain reaction-based method for rapidly identifying messenger ribonucleic acids whose levels increase or decrease, was performed with the nine different anchoring primers (oligodeoxythymidine11 **VN**: V = G, A, or C; N = G, A, or C) in combination with 24 different 10-base oligonucleotides of random sequence. The polymerase chain reaction products were separated by electrophoresis on a 5% polyacrylamide sequencing gel, and those whose levels changed were then cloned, sequenced, and compared with those in the GenBank database to determine whether they corresponded to a known sequence in the database or were novel. Semiquantitative reverse transcriptase-polymerase chain reaction was used to confirm differential expression of selected products. RESULTS: Messenger ribonucleic acid **differential display** revealed > 500 polymerase chain reaction products that were differentially expressed during gestation, 179 of which were cloned and sequenced. Of these, 157 were from messenger ribonucleic acids whose levels increased during gestation, and 22 were from transcripts that decreased. Eighty-seven (49%) were related to sequences in the GenBank database, of which 62 (35%) were from messenger ribonucleic acids encoding known proteins and 25 (14%) corresponded to known expressed sequence tags. The technique of semiquantitative reverse transcriptase-polymerase chain reaction confirmed the increased expression of messenger ribonucleic acids encoding beta-tropomyosin, type II phosphatidyl inositol-4-phosphate 5-kinase, and a novel myometrial messenger ribonucleic acid named RPU0901AC. CONCLUSION: Messenger ribonucleic acid **differential display** is a simple and sensitive method for rapidly identifying myometrial messenger ribonucleic acids that are differentially regulated during pregnancy. The identification of these differentially expressed messenger ribonucleic acids may lead to a better understanding of the molecular basis of normal and abnormal parturition.

L4 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 97411045 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9266082
TITLE: Automated **differential display** using a fluorescently labeled universal primer.
AUTHOR: Smith N R; Aldersley M; Li A; High A S; Moynihan T P; Markham A F; Robinson P A
CORPORATE SOURCE: Molecular Medicine Unit, St. James's University Hospital, Leeds, England, UK.
SOURCE: BioTechniques, (1997 Aug) 23 (2) 274-9.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Report; (TECHNICAL REPORT)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB We have modified the automated **differential display** reverse transcription polymerase chain reaction technique (DDRT-PCR) such that a single fluorescently labeled universal primer (d(F)CTCACG-GATCCGTCGATTTT) is used in all PCRs together with a selection of arbitrary primers. We term this fluorescent detection procedure FDDRT-PCR. Anchoring primers of general structure dTGGTCTCACGGATCCTCGA-(T)12 **VN** (where N can be any deoxynucleoside and V can be any deoxynucleoside other than thymidine) are used for the RT step, and the universal primer together with selected arbitrary primers are then used for the PCR amplification. Advantages of this approach are: (i) the fluorescently labeled universal primer is a constant feature in every PCR, so that changes in banding profile are highly likely to reflect the incorporation of different arbitrary 10-mer primers; (ii) artifacts that result from arbitrary 10-mer to arbitrary 10-mer primer amplifications are

not observed by fluoresence detection on an automated gene scanner because such products are not fluorescently labeled; (iii) sample throughput and ease of data handling are increased when compared with the conventional radioactive/manual approach and (iv) using a single fluorescently labeled primer in all PCRs is highly cost-effective.

L4 ANSWER 8 OF 8 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 97:113853 SCISEARCH
THE GENUINE ARTICLE: WE924
TITLE: DD/AP-PCR: Combination of **differential display** and arbitrarily primed PCR of oligo(dT) cDNA
AUTHOR: Rothschild C B (Reprint); Brewer C S; Bowden D W
CORPORATE SOURCE: WAKE FOREST UNIV, BOWMAN GRAY SCH MED, DEPT BIOCHEM, WINSTON SALEM, NC 27157 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: ANALYTICAL BIOCHEMISTRY, (1 FEB 1997) Vol. 245, No. 1, pp. 48-54.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0003-2697.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 11

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this report we describe the first direct comparison of **differential display** (DD) and arbitrarily primed PCR (AP-PGR) amplification of oligo(dT)-primed cDNA. Our results indicate that both of these widely used RNA fingerprinting techniques have their respective advantages and limitations. DD produces profiles specific to the anchored oligo(dT) primer used for cDNA synthesis. AP-PCR displays significant redundancy of profiles generated from different oligo(dT) cDNA pools, but is not as biased to the isolation of AT-rich or 3' sequences. It was found that both techniques can utilize cDNA synthesized using a generic anchored oligo(dT) primer (dT(12)**VN**; equimolar amounts of dT(12)VA, dT(12)VC, dT(12)VG, and dT(12)VT, where V is dA, dC, or dG); this efficiently selects for poly(A)(+) sequences from total RNA, and significantly reduces the number of cDNA preparations required per experiment. Using dT(12)**VN** cDNA pools generated from rat liver, spleen, and brain, the two approaches (AP-PCR and DD) were used in combination. Several known mRNAs were identified; some were unique to either technique and some were common to both. Since it is the RNA which is usually the limiting resource, maximum utilization may be achieved by generating a single pool of dT(12)**VN**-primed cDNA and performing both AP-PCR and DD (DD/AP-PCR). (C) 1997 Academic Press.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN###
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)
L3 20 S DIFFERENTIAL DISPLAY AND VN###
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)

=> s VNNN
L5 5 VNNN

=> d ibib abs 15 1-4

L5 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17928 BIOTECHDS
TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a oligo-dT primer of a specific sequence and a detectable marker at its 5' end; gene expression identification an characterization, database and computer bioinformatic software
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC
PATENT INFO: WO 2002036828 10 May 2002
APPLICATION INFO: WO 2000-US45401 1 Nov 2000
PRIORITY INFO: US 2000-244933 1 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-508123 [54]
AN 2002-17928 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific oligo-dT primer sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the VNx portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more

identimers comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I) Tn-**VNNN** (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L5 ANSWER 2 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 84:266183 SCISEARCH
THE GENUINE ARTICLE: SR533
TITLE: ELECTRON-PARAMAGNETIC-RES INVESTIGATION OF THE INCOMMENSURATE PHASE OF THBR4 WITH THE HELP OF THE GD3& **VNNN** BR-
AUTHOR: EMERY J (Reprint); HUBERT S; FAYET J C
CORPORATE SOURCE: FAC SCI LE MANS, SPECTROSCOPIE SOLIDE LAB, ERA 682, F-72017 LE MANS, FRANCE (Reprint); INST PHYS NUCL, RADIOCHEM LAB, F-91406 ORSAY, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: FERROELECTRICS, (1984) Vol. 53, No. 1-4, pp. 245.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: PHYS
LANGUAGE: ENGLISH
REFERENCE COUNT: 3

L5 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:353661 CAPLUS
DOCUMENT NUMBER: 136:351378
TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches

INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.
PATENT ASSIGNEE(S): Genomic Solutions Inc., USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036828	A2	20020510	WO 2001-US45401	20011101
WO 2002036828	A3	20030227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002018000	A5	20020515	AU 2002-18000	20011101
US 2003108874	A1	20030612	US 2001-2536	20011101
PRIORITY APPLN. INFO.:			US 2000-244933P P	20001101
			WO 2001-US45401 W	20011101

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' en) linked to a nucleotide sequence VNx (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all **VNNN** combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (identimer) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the

size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

L5 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:365527 CAPLUS
DOCUMENT NUMBER: 134:374588
TITLE: Proposal of an extended t-J Hamiltonian for high-Tc cuprates from ab initio calculations on embedded clusters
AUTHOR(S): Calzado, Carmen J.; Malrieu, Jean-Paul
CORPORATE SOURCE: IRSAMC, Laboratoire de Physique Quantique, Universite Paul Sabatier, Toulouse, 31062, Fr.
SOURCE: Physical Review B: Condensed Matter and Materials Physics (2001), 63(21), 214520/1-214520/13
CODEN: PRBMDO; ISSN: 0163-1829
PUBLISHER: American Physical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of accurate ab initio calcns. on CupOq finite clusters, properly embedded in the Madelung potential of the infinite lattice, have been performed in order to determine the local effective interactions in the CuO2 planes of La2-xSrxCuO4 compds. The values of the first-neighbor interactions, magnetic coupling (JNN = 125 meV), and hopping integral (tNN = -555 meV) have been confirmed. Important addnl. effects are evidenced, concerning essentially the second-neighbor hopping integral tNNN = +110 meV, the displacement of a singlet toward an adjacent colinear hole, hSDabc = -80 meV, a non-negligible hole-hole repulsion VNN-**VNNN** = 0.8 eV, and a strong anisotropic effect of the presence of an adjacent hole on the values of the first-neighbor interactions. The dependence of JNN and tNN on the position of neighbor hole(s) has been rationalized from the two-band model and checked from a series of addnl. ab initio calcns. An extended t-J model Hamiltonian has been proposed on the basis of these results. It is argued that the here-proposed three-body effects may play a role in the charge/spin separation observed in these compds., i.e., in the formation and dynamic of stripes.
REFERENCE COUNT: 74 THERE ARE 74 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 13:51:31 ON 22 MAR 2005
L1 13 S OLIGO-DT AND VN###
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)
L3 20 S DIFFERENTIAL DISPLAY AND VN###
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)
L5 5 S VNNN

=> s l5 5 ibib abs
MISSING OPERATOR L5 5 IBIB
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> d l5 5 ibib abs

L5 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:746459 CAPLUS
DOCUMENT NUMBER: 134:35582
TITLE: Proposal of an extended t-J Hamiltonian for high-Tc

cuprates from ab initio calculations on embedded clusters
AUTHOR(S): Calzado, Carmen J.; Malrieu, Jean-Paul
CORPORATE SOURCE: Laboratoire de Physique Quantique, IRSAMC, Universite Paul Sabatier, Toulouse, 31062, Fr.
SOURCE: Los Alamos National Laboratory, Preprint Archive, Condensed Matter (2000) 1-28, arXiv:cond-mat/0010257, 18 Oct 2000
CODEN: LNCMFR
URL: http://xxx.lanl.gov/pdf/cond-mat/0010257
PUBLISHER: Los Alamos National Laboratory
DOCUMENT TYPE: Preprint
LANGUAGE: English
AB A series of accurate ab initio calcns. on CupOq finite clusters, properly embedded on the Madelung potential of the infinite lattice, have been performed in order to determine the local effective interactions in the CuO2 planes of La2-xSrxCuO4 compds. The values of the first-neighbor interactions, magnetic coupling (JNN=125 meV) and hopping integral(tNN=-555 meV), have been confirmed. Important addnl. effects are evidenced, concerning essentially the second-neighbor hopping integral tNNN=+110meV, the displacement of a singlet toward an adjacent collinear hole, hSDabc=-80 meV, a non-negligible hole-hole repulsion VNN-**VNNN**=0.8 eV and a strong anisotropic effect of the presence of an adjacent hole on the values of the first-neighbor interactions. The dependence of JNN and tNN on the position of neighbor hole(s) has been rationalized from the two-band model and checked from a series of addnl. ab initio calcns. An extended t-J model Hamiltonian has been proposed on the basis of these results. It is argued that the here-proposed three-body effects may play a role in the charge/spin separation observed in these compds., i.e., in the formation and dynamic of stripes.
REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 13:51:31 ON 22 MAR 2005
L1 13 S OLIGO-DT AND VN###
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)
L3 20 S DIFFERENTIAL DISPLAY AND VN###
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)
L5 5 S VNNN

=> s primer and (VNNN### or VN1N2###)
L6 2 PRIMER AND (VNNN### OR VN1N2###)

=> d ibib abs l6 1-2

L6 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17928 BIOTECHDS
TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a oligo-dT **primer** of a specific sequence and a detectable marker at its 5' end;
gene expression identification an characterization, database and computer bioinformatic software
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC
PATENT INFO: WO 2002036828 10 May 2002

APPLICATION INFO: WO 2000-US45401 1 Nov 2000
PRIORITY INFO: US 2000-244933 1 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-508123 [54]
AN 2002-17928 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific oligo-dT **primer** sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an oligo-dT **primer** of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the VNx portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an oligo-dT **primer** of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I) Tn-**VNNN** (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The

system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L6 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:353661 CAPLUS
DOCUMENT NUMBER: 136:351378
TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches
INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.
PATENT ASSIGNEE(S): Genomic Solutions Inc., USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2002036828	A2	20020510	WO 2001-US45401		20011101
WO 2002036828	A3	20030227			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG					
AU 2002018000	A5	20020515	AU 2002-18000		20011101
US 2003108874	A1	20030612	US 2001-2536		20011101
PRIORITY APPLN. INFO.:			US 2000-244933P	P	20001101
			WO 2001-US45401	W	20011101

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where

desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' en) linked to a nucleotide sequence VNx (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all **VNNN** combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled **primer** (identimer) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

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=> d his

     (FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 13:51:31 ON 22 MAR 2005
L1           13 S OLIGO-DT AND VN###
L2            7 DUP REM L1 (6 DUPLICATES REMOVED)
L3           20 S DIFFERENTIAL DISPLAY AND VN###
L4            8 DUP REM L3 (12 DUPLICATES REMOVED)
L5            5 S VNNN
L6            2 S PRIMER AND (VNNN### OR VN1N2###)

=> s vn1n2#
L7            0 VN1N2#

=> identimer
IDENTIMER IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s identimer
L8            2 IDENTIMER
```

=> d ibib abs l8 1-2

L8 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17928 BIOTECHDS
TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an **identimer** having a oligo-dT primer of a specific sequence and a detectable marker at its 5' end;
gene expression identification an characterization, database and computer bioinformatic software
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC
PATENT INFO: WO 2002036828 10 May 2002
APPLICATION INFO: WO 2000-US45401 1 Nov 2000
PRIORITY INFO: US 2000-244933 1 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-508123 [54]
AN 2002-17928 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an **identimer** having a specific oligo-dT primer sequence, where the **identimer** comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an **identimer** comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I) or (II), where the **identimer** also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the **identimer** so that the polyT portion of the **identimer** hybridizes to the polyA tail of the mRNA and the VNx portion of the **identimer** hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the **identimer**; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the **identimer** to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an **identimer** comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the **identimer** so that the polyT portion of the **identimer** hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the **identimer** hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the **identimer**; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an **identimer** comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the **identimer** so that the polyT portion of

the **identimer** hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the **identimer** hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the **identimer**; (5) a kit comprising: (a) one or more identimers comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I), where the **identimer** also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

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USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

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EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L8 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER:	2002:353661 CAPLUS
DOCUMENT NUMBER:	136:351378
TITLE:	Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches
INVENTOR(S):	Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.
PATENT ASSIGNEE(S):	Genomic Solutions Inc., USA
SOURCE:	PCT Int. Appl., 45 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 2002036828 A2 20020510 WO 2001-US45401 20011101
WO 2002036828 A3 20030227
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
AU 2002018000 A5 20020515 AU 2002-18000 20011101
US 2003108874 A1 20030612 US 2001-2536 20011101
PRIORITY APPLN. INFO.: US 2000-244933P P 20001101
WO 2001-US45401 W 20011101

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' en) linked to a nucleotide sequence VNx (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all VNNN combinations of the **identimer**, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. **identimer**), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (**identimer**) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "*" at the 5' end of the **identimer** in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by **identimer** priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

=> s VN? and permutation
L9 19 VN? AND PERMUTATION

=> dup rem 19
PROCESSING COMPLETED FOR L9

L10 7 DUP REM L9 (12 DUPLICATES REMOVED)

=> d ibib abs l10 1-10

L10 ANSWER 1 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:852766 SCISEARCH
THE GENUINE ARTICLE: BAV32
TITLE: Particle swarm optimization algorithm for **permutation** flowshop sequencing problem
AUTHOR: Tasgetiren M F (Reprint); Sevkli M; Liang Y C; Gencyilmaz G
CORPORATE SOURCE: Fatih Univ, Dept Management, TR-34500 Istanbul, Turkey (Reprint); Fatih Univ, Dept Ind Engn, TR-34500 Istanbul, Turkey; Yuan Ze Univ, Dept Ind Engn & Management, Taoyuan 320, Taiwan; Istanbul Kultur Univ, Dept Management, Istanbul, Turkey
COUNTRY OF AUTHOR: Turkey; Taiwan
SOURCE: ANT COLONY OPTIMIZATION AND SWARM INTELLIGENCE, PROCEEDINGS, (OCT 2004) Vol. 3172, pp. 382-389. Publisher: SPRINGER-VERLAG BERLIN, HEIDELBERGER PLATZ 3, D-14197 BERLIN, GERMANY.
ISSN: 0302-9743.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 20
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This paper presents a particle swarm optimization algorithm (PSO) to solve the **permutation** flowshop sequencing problem (PFSP) with makespan criterion. Simple but very efficient local search based on the variable neighborhood search (**VNS**) is embedded in the PSO algorithm to solve the benchmark suites in the literature. The results are presented and compared to the best known approaches in the literature. Ultimately, a total of 195 out of 800 best-known solutions in the literature is improved by the **VNS** version of the PSO algorithm.

L10 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
ACCESSION NUMBER: 2002:294993 BIOSIS
DOCUMENT NUMBER: PREV200200294993
TITLE: Genetic diversity among populations and size classes of buckeyes (Aesculus: Hippocastanaceae) examined with multilocus **VNTR** probes.
AUTHOR(S): Lim, H. W. [Reprint author]; Pelikan, S. [Reprint author]; Rogstad, S. H. [Reprint author]
CORPORATE SOURCE: Biological Sciences, University of Cincinnati, ML0006, Cincinnati, OH, 45221-0006, USA
steven.rogstad@uc.edu
SOURCE: Plant Systematics and Evolution, (2002) Vol. 230, No. 3-4, pp. 125-141. print.
CODEN: ESPFBP. ISSN: 0378-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 May 2002
Last Updated on STN: 15 May 2002

AB Little is known about genetic variation in members of the genus Aesculus (Hippocastanaceae), in particular A. flava (yellow buckeye) and A. glabra (Ohio buckeye). Here, three synthetic DNA probes (composed of tandemly repeated, core sequences) that reveal alleles at multiple variable-number tandem-repeat (**VNTR**) loci in these two species were used to investigate: 1) levels of genetic variation in one stand of A. flava and three isolated stands of A. glabra; 2) whether the stands of A. glabra are genetically differentiated from one another; 3) whether there has been selection for more heterozygous individuals through time in one stand each

of A. flava and A. glabra; and 4) whether a possible genetic bottleneck had occurred during the formation of either species of Aesculus. First, variation of **VNTR** genetic markers within and among three populations of A. glabra separated by 60-180 km was examined. In each one hectare (ha) population, 22 individuals were randomly sampled. Among the three populations, the mean number of bands scored per individual was 80.35 and the average number of estimated loci surveyed was 54.17. Mean similarity and estimated heterozygosity within populations ranged from 0.634 to 0.743 and from 0.342 to 0.486, respectively. The mean similarity across populations was 0.657, while the mean estimated heterozygosity across populations was 0.484 for A. glabra. The most isolated site was the most genetically differentiated as indicated by differences in levels of similarity, heterozygosity, and Fst value comparisons. In a separate experiment, genetic variation in 22 large (reproductively mature; dbh > 8 cm) individuals was compared with that in 22 small (not yet reproductive; dbh < 1 cm) individuals collected within one ha stands for both A. flava and A. glabra. Mean similarity values among large versus small individuals of A. flava were 0.665 versus 0.662, while for A. glabra the corresponding values were 0.686 versus 0.691, respectively. **Permutation** tests of these similarity data detected no evidence for size class genetic differentiation in either species (both p-values > 0.050). Further, **permutation** tests for the number of bands per individual (average band number should be higher in more heterozygous individuals) detected no significant differences between size classes for either species. Thus, evidence of pronounced inbreeding and/or selection altering population genetics within small relative to large individuals was not detected. In addition, comparable similarity and heterozygosity values between these two closely related species (which still maintain an active zone of hybridization) suggests that either: 1) no extreme genetic bottleneck has accompanied the formation of these species from a common ancestor; or 2) signs of such a bottleneck have largely been eliminated. These studies demonstrate the utility of multilocus **VNTR** DNA probes for investigating genetic variation within and among plant populations, between size classes within a population, and between closely related species.

L10 ANSWER 3 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:9602 SCISEARCH
THE GENUINE ARTICLE: 502JE
TITLE: Linear maps leaving the alternating group invariant
AUTHOR: Chiang H; Li C K (Reprint)
CORPORATE SOURCE: Coll William & Mary, Dept Math, POB 8795, Williamsburg, VA 23187 USA (Reprint); Coll William & Mary, Dept Math, Williamsburg, VA 23187 USA
COUNTRY OF AUTHOR: USA
SOURCE: LINEAR ALGEBRA AND ITS APPLICATIONS, (1 JAN 2002) Vol. 340, pp. 69-80.
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010 USA.
ISSN: 0024-3795.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 3

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Let A(n) be the group of n x n even **permutation** matrices, and let **Vn** be the real linear space spanned by A(n). The purpose of this note is to characterize those linear operators phi on V-n satisfying phi (A(n)) = A(n). This answers a question raised by C.K. Li, B.S. Tam, N.K. Tsing [Linear Algebra Appl., to appear]. (C) 2002 Elsevier Science Inc. All rights reserved.

L10 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2

ACCESSION NUMBER: 1999:493524 BIOSIS
DOCUMENT NUMBER: PREV199900493524
TITLE: Genetic diversity of Typha latifolia (Typhaceae) and the impact of pollutants examined with tandem-repetitive DNA probes.
AUTHOR(S): Keane, Brian; Pelikan, Stephan; Toth, Greg P.; Smith, M. Kate; Rogstad, Steven H. [Reprint author]
CORPORATE SOURCE: Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH, 45221-0006, USA
SOURCE: American Journal of Botany, (Sept., 1999) Vol. 86, No. 9, pp. 1226-1238. print.
CODEN: AJBOAA. ISSN: 0002-9122.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 1999
Last Updated on STN: 16 Nov 1999

AB Genetic diversity at variable-number-tandem-repeat (**VNTR**) loci was examined in the common cattail, Typha latifolia (Typhaceae), using three synthetic DNA probes composed of tandemly repeated "core" sequences (GACA, GATA, and GCAC). The principal objectives of this investigation were to determine whether: (1) the previously reported almost complete lack of polymorphism at allozyme loci in this species was indicative of a reduced amount of genetic diversity at **VNTR** loci as well; (2) **VNTR** markers were informative about possible clonal propagation; and (3) significant differences in genetic structure of sampling sites were associated with differences in environmental levels of pollutants at those sites. Previously, widespread sampling across the eastern United States, surveying across ten allozyme loci, has detected only two genotypes, involving a difference at a single locus, among 104 populations. In this study, the amount of genetic diversity detected at **VNTR** loci: (1) among ramets (N= 40; 40 genotypes detected) collected at apprx8-km intervals along a 320-km transect; (2) among ramets (N = 220; 117 genotypes detected) from five study sites separated by 50-3000 m; and (3) even among ramets within each study site (N = 44 per site; from 13 to 34 genotypes detected per site (270 m2)) exceeds that previously found in those more geographically widespread allozyme surveys. Among the 260 ramets analyzed here, the mean number of bands scored per individual was 48.61 (SD = 2.80). Mean genetic similarity among ramets collected along the 320-km transect was 0.91, which was within the range of mean genetic similarity within the five study sites (range: 0.89-0.95). Among the five study sites, 61% of the samples analyzed appeared to be clonal ramets, with up to 12 clones detected for 44 ramets sampled within a site. Clones grew intermingled and ranged up to 39 m in extent. **Permutation** tests of genetic similarity revealed significant genetic differentiation between each of the five study sites. Consistent with the previous allozyme studies, T. latifolia was characterized by extremely low genetic variation relative to levels of polymorphism detected at **VNTR** loci in other plant species. Estimated heterozygosity among ramets along the 320-km transect ranged from 0.11 to 0.13, while that within the five study sites ranged from 0.05 to 0.12. Estimates of Fst (0.32-0.41) also indicated considerable genetic subdivision among these stands. Significantly higher genetic diversity was detected at the two study sites that chemistry and toxicity data indicate to be the most severely impacted by pollutants. Although this correlation does not establish cause and effect, the results of this study indicate that the analysis of genetic diversity at **VNTR** loci may be a useful tool for monitoring anthropogenic-induced changes in the genetic structure of natural populations of plants.

L10 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 3
ACCESSION NUMBER: 1998:93708 BIOSIS
DOCUMENT NUMBER: PREV199800093708
TITLE: Genetic diversity of Philippine Rubus moluccanus L.

(Rosaceae) populations examined with **VNTR** DNA probes.

AUTHOR(S): Busemeyer, Daniel T.; Pelikan, Stephan; Kennedy, Robert S.; Rogstad, Steven H. [Reprint author]

CORPORATE SOURCE: Dep. Biol. Sci. ML6, Univ. Cincinnati, Cincinnati, OH 45221-0006, USA

SOURCE: Journal of Tropical Ecology, (Nov., 1997) Vol. 13, No. 6, pp. 867-884. print.
ISSN: 0266-4674.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Feb 1998
Last Updated on STN: 25 Feb 1998

AB Two synthetic DNA probes composed of tandemly repeated 'core' sequences (GACA and GATA) were used to examine genetic diversity at multiple variable-number-tandem-repeat (**VNTR**) loci within and among four spatially isolated Philippine populations of Rubus moluccanus L. (Rosaceae), an uncultivated bramble widely distributed throughout southeast Asia - Malesia. Central goals were to determine whether apomictic propagation was detectable in R. moluccanus and to examine whether populations isolated on separate islands, or on mountain tops on a single island, were genetically differentiated. Sampling 22 individuals per population, the findings include: (1) no two individuals shared identical **VNTR** band profiles and thus apomictic propagation was not detected; (2) the mean number of bands scored per individual was 24.3 (SD = 3.91) and the proportion of polymorphic loci within populations ranged from 0.69 to 1.00 (mean = 0.86 +- SD = 0.085); (3) the average proportion of bands shared between individuals within populations ranged from 0.39 to 0.67 (0.50 +- 0.067), while average interpopulation similarity ranged from 0.21 to 0.50 (0.32 +- 0.092); and (4) estimated heterozygosity within populations ranged from 0.42 to 0.79 (0.62 +- 0.083), while interpopulation heterozygosity ran from 0.62 to 0.81 (0.74 +- 0.062). **Permutation** tests were used to estimate the statistical significance of differences in similarity between populations. A Luzon population isolated by 1200 km was always significantly different in similarity tests when compared with each of the other three populations, all located on Mindanao (and separated by at least 100 km but less than 250 km). Of the latter three, only one population differed significantly from the other two in the degree of **VNTR** markers shared, possibly reflecting biogeographic partitioning suggested for the island. Across all populations, average estimated Fst was 0.154, although mean inter-island Fst (0.224) was significantly higher than mean intra-island Fst (0.085). These results suggest that, while intra-island gene flow is relatively high, significant differentiation of tropical species may occur even over short distances on individual islands. Preservation of only a limited number of populations may result in a significant loss of genetic diversity in such species.

L10 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 97124707 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8969844

TITLE: GELSTATS: a computer program for population genetics analyses using **VNTR** multilocus probe data.

AUTHOR: Rogstad S H; Pelikan S
CORPORATE SOURCE: University of Cincinnati, OH, USA.. rogstad@ucbeh.san.uc.edu

SOURCE: BioTechniques, (1996 Dec) 21 (6) 1128-31.
Journal code: 8306785. ISSN: 0736-6205.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970321

Last Updated on STN: 19970321
Entered Medline: 19970311

AB GELSTATS, a computer program for population genetics analyses utilizing genetic markers revealed with variable number tandem repeat (**VNTR**) multilocus probes, is described and made available (both as C++ source code and as an executable DOS program). The program calculates several population genetics parameters, including: (i) individual and population band numbers; (ii) population bands exhibiting complete linkage (redundant examples of such bands can be removed in subsequent analyses); (iii) similarity (fraction of bands shared) between individuals and average similarity within and between designated groups; (iv) estimated probability that two individuals chosen at random will have identical band profiles; (v) heterozygosity estimates for designated groups; and (vi) Fst estimates. Nonparametric **permutation** methods are used to assess the significance of differences in both within- and between-group similarity. A jackknife test for heterozygosity differences between groups is also computed. Examples of GELSTATS analyses illustrate some features of the program.

L10 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 93170644 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8094698
TITLE: Intraclass and interclass correlations of allele sizes within and between loci in DNA typing data.
AUTHOR: Chakraborty R; Srinivasan M R; de Andrade M
CORPORATE SOURCE: Center for Demographic and Population Genetics, University of Texas Graduate School of Biomedical Sciences, Houston 77225.
CONTRACT NUMBER: GM-41399 (NIGMS)
SOURCE: Genetics, (1993 Feb) 133 (2) 411-9.
Journal code: 0374636. ISSN: 0016-6731.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19950206
Entered Medline: 19930323

AB Nonparametric measures of correlations of DNA fragment lengths within and between variable number of tandem repeat (**VNTR**) loci are proposed to test the hypothesis of random association of allele sizes at **VNTR** loci. Transformations of these nonparametric correlation measures are suggested to detect deviations of their null expectations caused by population subdivision and errors of measurement of **VNTR** fragment lengths. Analytic and **permutation**-based computer simulation studies are performed to show that under the hypothesis of independence of allele sizes the transformed correlation measures are normally distributed, irrespective of the **VNTR** fragment size distribution in the population even when the number of individuals samples is as low as 100. Power calculations are performed to establish that the current population data on six **VNTR** loci in the US Hispanic sample are in accordance with the hypothesis of random association of allele sizes within and between loci. Implications of these results in the context of forensic use of DNA typing are also discussed.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 13:51:31 ON 22 MAR 2005
L1 13 S OLIGO-DT AND VN###

```
L2             7 DUP REM L1 (6 DUPLICATES REMOVED)
L3            20 S DIFFERENTIAL DISPLAY AND VN###
L4             8 DUP REM L3 (12 DUPLICATES REMOVED)
L5             5 S VNNN
L6             2 S PRIMER AND (VNNN### OR VN1N2###)
L7             0 S VN1N2#
L8             2 S IDENTIMER
L9            19 S VN? AND PERMUTATION
L10            7 DUP REM L9 (12 DUPLICATES REMOVED)

=> s VNx
L11          185 VNX

=> s l11 and oligo-dt
   3 FILES SEARCHED...
L12            1 L11 AND OLIGO-DT

=> d all

L12   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN    2002-17928  BIOTECHDS
TI    Identifying and characterizing gene expression in samples, for
      identifying mRNAs expressed at different levels, comprises employing an
      identimer having a oligo-dT primer of a specific
      sequence and a detectable marker at its 5' end;
         gene expression identification an characterization, database and
         computer bioinformatic software
AU    KANE M D; DOMBKOWSKI A A; NAGEL A C
PA    GENOMIC SOLUTIONS INC
PI    WO 2002036828 10 May 2002
AI    WO 2000-US45401 1 Nov 2000
PRAI  US 2000-244933 1 Nov 2000
DT    Patent
LA    English
OS    WPI: 2002-508123 [54]
AB    DERWENT ABSTRACT:
      NOVELTY - Systems for identification and characterization of gene
      expression in one or more samples, comprise an identimer having a
      specific oligo-dT primer sequence, where the
      identimer comprises a detectable marker at its 5' end.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
      following: (1) a system (M1) for identification and characterization of
      gene expression in one or more samples, comprising: (a) providing one or
      more samples comprising one or more mRNA molecules; (b) providing an
      identimer comprising an oligo-dT primer of sequence,
      from 5' to 3' end, of (I) or (II), where the identimer also comprises a
      detectable marker at its 5' end; (c) contacting the mRNA with the
      identimer so that the polyT portion of the identimer hybridizes to the
      polyA tail of the mRNA and the VNx portion of the identimer
      hybridizes with portions of the mRNA immediately upstream of the polyA
      tail; (d) reverse transcribing the mRNA to produce a first strand cDNA
      that includes the identimer; (e) synthesizing a second DNA strand
      complementary to the first strand cDNA to form a duplex; (f) cleaving the
      duplex with a sequence-specific cleaving agent to provide one or more
      duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA
      polymerase promoter site to one or more of the cleavage fragments; and
      (h) amplifying one or more ligated cleavage fragments using the identimer
      to produce one or more amplified fragments comprising sequences
      complementary to a 3' end of the mRNA; (2) a system for identification
      and characterization of gene expression in one or more samples, by: (a)
      employing steps (a) - (c) of M1; (b) providing a second sample comprising
      one or more mRNA molecules; (c) providing an identimer comprising (I) or
      (II); and (d) employing steps (c)-(h) of M1; (3) a system for
      identification and characterization of gene expression in one or more
```

samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-**VNx** (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

CC GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; BIOINFORMATICS and ANALYSIS, Software; BIOINFORMATICS and ANALYSIS, Databases

CT GENE EXPRESSION IDENTIFICATION, CHARACTERIZATION, IDENTIMER, **OLIGO-DT** DNA PRIMER SEQUENCE, DETECTABLE MARKER, RNA SAMPLE, RNA-POLYMERASE PROMOTER, CLEAVAGE FRAGMENT ASSOCIATED GENE IDENTIFICATION, DATABASE, COMPUTER BIOINFORMATIC SOFTWARE BIOINFORMATICS

(21, 49)

=> poly-dT and VN####
POLY-DT IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> spoly-dT and VN####
SPOLY-DT IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s poly-dT and VN####
L13 4 POLY-DT AND VN####

=> d ibib abs l13 1-4

L13 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 90196009 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2316523
TITLE: Five polymorphic microsatellite **VNTRs** on the human X chromosome.
AUTHOR: Luty J A; Guo Z; Willard H F; Ledbetter D H; Ledbetter S; Litt M
CORPORATE SOURCE: Department of Biochemistry, Oregon Health Sciences University, Portland 97201-3098.
CONTRACT NUMBER: HD20619 (NICHD)
R01-GM32500 (NIGMS)
SOURCE: American journal of human genetics, (1990 Apr) 46 (4) 776-83.
Journal code: 0370475. ISSN: 0002-9297.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900423

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n = approximately 10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These "microsatellite" variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately 25% of the phage clones hybridized to a **poly** (**dT**-dG).poly(dA-dC) probe. Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of "classical" **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs**.

on STN
ACCESSION NUMBER: 90113454 EMBASE
DOCUMENT NUMBER: 1990113454
TITLE: Five polymorphic microsatellite **VNTRs** on the human X chromosome.
AUTHOR: Luty J.A.; Guo Z.; Willard H.F.; Ledbetter D.H.; Ledbetter S.; Litt M.
CORPORATE SOURCE: Department of Biochemistry, Oregon Health Sciences, University,Portland, OR 97201-3098, United States
SOURCE: American Journal of Human Genetics, (1990) 46/4 (776-783). ISSN: 0002-9297 CODEN: AJHGAG
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n = .apprx.10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These 'microsatellite' variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately 25% of the phage clones hybridized to a **poly** (**dT**-dG).poly(dA-dC) probe. Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of 'classical' **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs.**

L13 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1990:261676 BIOSIS
DOCUMENT NUMBER: PREV199090003762; BA90:3762
TITLE: FIVE POLYMORPHIC MICROSATELLITE **VNTRS** ON THE HUMAN X CHROMOSOME.
AUTHOR(S): LUTY J A [Reprint author]; GUO Z; WILLARD H F; LEDBETTER D H; LEDBETTER S; LITT M
CORPORATE SOURCE: DEP BIOCHEMISTRY, OREGON HEALTH SCIENCES UNIV, PORTLAND, OREG 97201-3098, USA
SOURCE: American Journal of Human Genetics, (1990) Vol. 46, No. 4, pp. 776-783.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 5 Jun 1990
Last Updated on STN: 5 Jun 1990

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT · dG/dA · dC)n, where n = .apprx. 10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These "microsatellite" variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately

25% of the phage clones hybridized to a **poly** (**dT**
-dG)-poly(dA-dC) probe. Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of "classical" **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs.**

L13 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1990:401430 CAPLUS
DOCUMENT NUMBER: 113:1430
TITLE: Five polymorphic microsatellite **VNTRs** on the human X chromosome
AUTHOR(S): Luty, J. A.; Guo, Z.; Willard, H. F.; Ledbetter, D. H.; Ledbetter, S.; Litt, M.
CORPORATE SOURCE: Dep. Biochem., Oregon Health Sci. Univ., Portland, OR, USA
SOURCE: American Journal of Human Genetics (1990), 46(4), 776-83
CODEN: AJHGAG; ISSN: 0002-9297
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The human genome contains approx. 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n = .apprx.10-60. Several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These microsatellite variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, a flow-sorted X chromosome-specific genomic library was screened for microsatellites. Approx. 25% of the phage clones hybridized to a **poly** (**dT**-dG).poly(dA-dC) probe. Of seven X-linked microsatellites present in pos. phages, 5 are polymorphic and 3 have both 8 or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, the X localization of these **VNTRS** was confirmed and 4 of them were regionally mapped. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Center du Polymorphisme Humain families. It was concluded that whatever factors limit the occurrence of classical **VNTRs** and RFLPs on the X chromosome they do not appear to operate in the case of microsatellite **VNTRs.**

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 13:51:31 ON 22 MAR 2005
L1 13 S OLIGO-DT AND VN###
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)
L3 20 S DIFFERENTIAL DISPLAY AND VN###
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)
L5 5 S VNNN
L6 2 S PRIMER AND (VNNN### OR VN1N2###)
L7 0 S VN1N2#
L8 2 S IDENTIMER
L9 19 S VN? AND PERMUTATION
L10 7 DUP REM L9 (12 DUPLICATES REMOVED)
L11 185 S VNX

```
L12             1 S L11 AND OLIGO-DT
L13             4 S POLY-DT AND VN####

=> s poly-dT and VNNN
L14             0 POLY-DT AND VNNN

=>
=> polyadenylation an VN?
POLYADENYLATION IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s polyadenylation an VN?
L15             0 POLYADENYLATION AN VN?

=> d his

     (FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 13:51:31 ON 22 MAR 2005
L1             13 S OLIGO-DT AND VN###
L2              7 DUP REM L1 (6 DUPLICATES REMOVED)
L3             20 S DIFFERENTIAL DISPLAY AND VN###
L4              8 DUP REM L3 (12 DUPLICATES REMOVED)
L5              5 S VNNN
L6              2 S PRIMER AND (VNNN### OR VN1N2###)
L7              0 S VN1N2#
L8              2 S IDENTIMER
L9             19 S VN? AND PERMUTATION
L10             7 DUP REM L9 (12 DUPLICATES REMOVED)
L11           185 S VNX
L12             1 S L11 AND OLIGO-DT
L13             4 S POLY-DT AND VN####
L14             0 S POLY-DT AND VNNN
L15             0 S POLYADENYLATION AN VN?

=> s l11 and (primer  or oligo-dT)
L16             5 L11 AND (PRIMER OR OLIGO-DT)

=> dup rem l15 1-5 ibib abs
L15 HAS NO ANSWERS

=> d ibib abs l16 1-5

L16  ANSWER 1 OF 5     MEDLINE on STN
ACCESSION NUMBER:   2003545638     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14624033
TITLE:              Effects of vomeronasal organ removal on the sperm motility
                    in male mice.
AUTHOR:             Koyama Sachiko; Kamimura Shinji
CORPORATE SOURCE:   Division of Biology, Department of Life Sciences, Graduate
                    School of Arts & Sciences, University of Tokyo, Meguro-ku,
                    Tokyo, Japan.. sakoyama@indiana.edu
SOURCE:             Zoological science, (2003 Nov) 20 (11) 1355-8.
                    Journal code: 8702287. ISSN: 0289-0003.
PUB. COUNTRY:       Japan
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200402
ENTRY DATE:         Entered STN: 20031120
                    Last Updated on STN: 20040211
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Entered Medline: 20040210

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17928 BIOTECHDS
TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a **oligo-dT primer** of a specific sequence and a detectable marker at its 5' end; gene expression identification an characterization, database and computer bioinformatic software
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC
PATENT INFO: WO 2002036828 10 May 2002
APPLICATION INFO: WO 2000-US45401 1 Nov 2000
PRIORITY INFO: US 2000-244933 1 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-508123 [54]
AN 2002-17928 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific **oligo-dT primer** sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an **oligo-dT primer** of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the **VNx** portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more

samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT primer** of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-**VNx** (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L16 ANSWER 3 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:111479 BIOSIS
DOCUMENT NUMBER: PREV200400112568
TITLE: Effects of vomeronasal organ removal on the sperm motility in male mice.
AUTHOR(S): Koyama, Sachiko [Reprint Author]; Kamimura, Shinji

CORPORATE SOURCE: Department of Chemistry, Institute of Pheromone Research, Indiana University, 800E Kirkwood Ave., Bloomington, IN, 47401, USA
sakoyama@indiana.edu
SOURCE: Zoological Science (Tokyo), (November 2003) Vol. 20, No. 11, pp. 1355-1358. print.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Feb 2004
Last Updated on STN: 25 Feb 2004

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:66783 SCISEARCH
THE GENUINE ARTICLE: 759TA
TITLE: Effects of vomeronasal organ removal on the sperm motility in male mice
AUTHOR: Koyama S (Reprint); Kamimura S
CORPORATE SOURCE: Indiana Univ, Dept Chem, Inst Pheromone Res, 800E Kirkwood Ave, Bloomington, IN 47401 USA (Reprint); Univ Tokyo, Grad sch Arts & Sci, Dept Life Sci, Div Biol, Meguro Ku, Tokyo 1538902, Japan
COUNTRY OF AUTHOR: USA; Japan
SOURCE: ZOOLOGICAL SCIENCE, (NOV 2003) Vol. 20, No. 11, pp. 1355-1358.
Publisher: ZOOLOGICAL SOC JAPAN, TOSHIN-BUILDING, HONGO 2-27-2, BUNKYO-KU, TOKYO, 113, JAPAN.
ISSN: 0289-0003.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 15
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:353661 CAPLUS
DOCUMENT NUMBER: 136:351378
TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches
INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.
PATENT ASSIGNEE(S): Genomic Solutions Inc., USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2002036828	A2	20020510	WO 2001-US45401		20011101
WO 2002036828	A3	20030227			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG					
AU 2002018000	A5	20020515	AU 2002-18000		20011101
US 2003108874	A1	20030612	US 2001-2536		20011101
PRIORITY APPLN. INFO.:			US 2000-244933P	P	20001101
			WO 2001-US45401	W	20011101

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' en) linked to a nucleotide sequence **VNx** (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all VNNN combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled **primer** (identimer) to enable the detection of all 3' cDNA

fragments for size and abundance (fluorescence label is denoted as an "*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

=> end
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-5.84	-5.84

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